

M. Bodanszky · A. Bodanszky

The Practice of Peptide Synthesis



Springer-Verlag
Berlin Heidelberg New York Tokyo 1984

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ISBN 3-540-13471-9 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-13471-9 Springer-Verlag New York Heidelberg Berlin Tokyo

Library of Congress Cataloging in Publication Data

Bodanszky, Miklos. The practice of peptide synthesis. (Reactivity and structure: concepts in organic chemistry; v. 21). Includes index. I. Peptide synthesis. I. Bodanszky, A. (Agnes), 1925-. II. Title. III. Series: Reactivity and structure; v. 21. QD431.B763 1984 547.7'56 84-5421

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Printed in Germany

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Printing: Brüder Hartmann, Berlin. Bookbinding: Lüderitz & Bauer, Berlin.
2152/3020-543210

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Reactivity and Structure Concepts in Organic Chemistry

Volume 21

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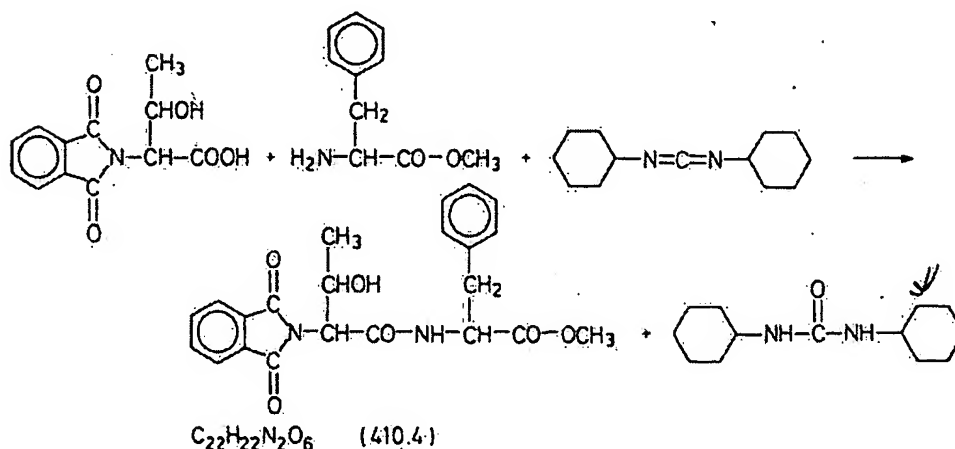
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G. Peptide Bond Formation with the Aid of Coupling Reagents

1. The Dicyclohexylcarbodiimide (DCC, DCCI) Method [1]

a. Coupling with DCC

N-Phthaloyl-L-threonyl-L-phenylalanine Methyl Ester [2]



A solution of L-phenylalanine methyl ester hydrochloride (5.4 g, 25 mmol) in water (20 ml) is treated with a solution of K_2CO_3 (5.0 g, 36 mmol) in water (10 ml) and the mixture is extracted with ether (three times, 25 ml each time). The ether extracts are pooled, dried over MgSO_4 and the solvent removed under moderately reduced pressure with the help of a water aspirator. The residue [3] (about 3.6 g, 20 mmol) is added to a solution of *N*-phthaloyl-L-threonine [4] (2.53 g, 10 mmol) in dichloromethane [5] (40 ml) followed by the addition of dicyclohexylcarbodiimide [6, 7] (2.06 g, 10 mmol). A precipitate, *N,N'*-dicyclohexylurea, starts to separate almost immediately and its amount gradually increases. After five hours at room temperature the urea derivative is removed by filtration [8] and washed with dichloromethane (20 ml). The combined filtrate and washings are extracted with N HCl (30 ml), N KHCO_3 (30 ml), water (30 ml), dried over MgSO_4 and evaporated to dryness *in vacuo*. Recrystallization of the residue from acetone-ether affords the pure dipeptide derivative (3.72 g, 91 %) melting at 149–152 °C. A sample is recrystallized for analysis from acetone-ether melts at 153–154 °C; $[\alpha]_D^{27} +1.9^\circ$ (c 0.7, dimethylformamide).

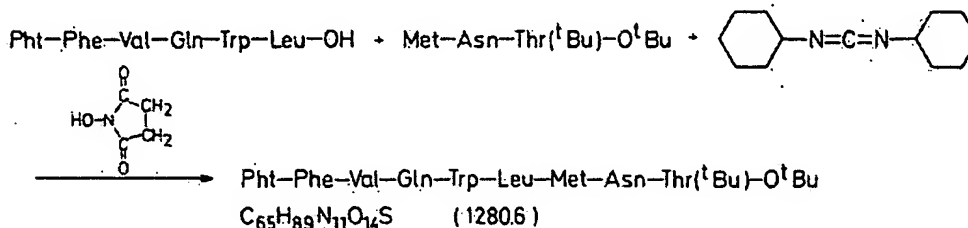
11. Activation and Coupling

1. Sheehan, J. C., Hess, G. P.: J. Amer. Chem. Soc. 77, 1067 (1955)
2. Sheehan, J. C., Goodman, M., Hess, G. P.: J. Amer. Chem. Soc. 78, 1367 (1956)
3. The free amino acid ester should not be stored: it is gradually transformed into the diketopiperazine *cyclo*-L-Phe-L-Phe-.
4. Hydroxyamino acids can be used without blocking the hydroxyl group, but with excess acylating agent, O-acylation can occur; cf. Bodanszky, M., Ondetti, M. A.: Chem. Ind. 1966, 26.
5. Preferably freshly distilled solvent should be used.
6. Equally good results can be obtained with diisopropylcarbodiimide.
7. Carbodiimides are known for causing allergic reactions. They should be handled with care; contact with the skin and particularly with the eyes should be avoided.
8. Removal of N,N'-dicyclohexylurea is usually incomplete; a small amount which remains in solution can contaminate the product and has to be removed from it by crystallization or chromatography. This difficulty can be circumvented by the application of water soluble carbodiimides since they give rise to water soluble urea derivatives. (cf. Sheehan, J. C., Hlavka, J. J.: J. Org. Chem. 21, 439 (1956)). The water soluble reagent N-ethyl-N'-3-dimethylaminopropylcarbodiimide hydrochloride is commercially available.

II. Activation and Coupling

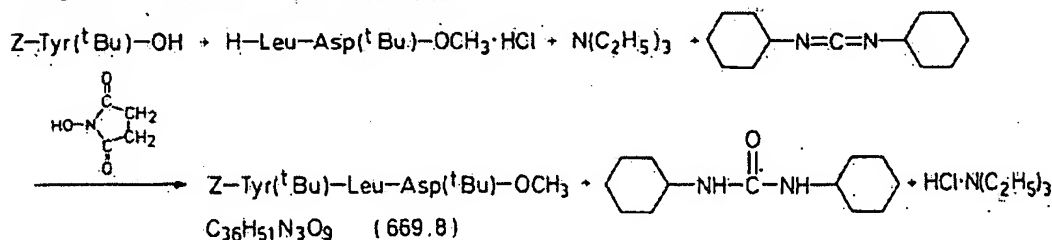
c. Coupling with Dicyclohexylcarbodiimide in the Presence of N-Hydroxysuccinimide [1]

Phthalyl-L-phenylalanyl-L-valyl-L-glutaminyl-L-tryptophyl-L-leucyl-L-methionyl-L-asparaginyl-O-tert.butyl-L-threonine tert.butyl Ester [2]



Phthalyl-L-phenylalanyl-L-valyl-L-glutaminyl-L-tryptophyl-L-leucine, hemihydrate (8.31 g, 10 mmol), L-methionyl-L-asparaginyl-O-*tert*.butyl-L-threonine *tert*.butyl ester (4.78 g, 10 mmol) and N-hydroxysuccinimide (1.15 g, 10 mmol) are dissolved in dimethylformamide (67 ml). The solution is stirred and cooled to -10°C during the addition of dicyclohexylcarbodiimide (2.06 g, 10 mmol). After two hours at -10°C and 48 hours at -3°C water (330 ml) is added [3] to the reaction mixture: the material which separates soon solidifies. It is collected on a filter, thoroughly washed with a saturated solution of NaHCO_3 in water (200 ml) then with water (200 ml) and dried over P_2O_5 *in vacuo*. The crude product (12.1 g) melts with decomposition at 225 to 226°C . After precipitation from an ethanolic solution with water the purified nonapeptide derivative (9.7 g, 75%) melts at 226 – 227°C ; $[\alpha]_D^{20} -31.8^\circ$ (c 1, dimethylformamide).

N-Benzoyloxycarbonyl-O-tert.butyl-L-tyrosyl-L-leucyl-β-tert.butyl-L-aspartic Acid Methyl Ester [2]



N-Benzoyloxycarbonyl-O-*tert*.butyl-L-tyrosine (3.72 g, 10 mmol) and L-leucyl-β-*tert*.butyl-L-aspartic acid methyl ester hydrochloride (3.54 g, 10 mmol), N-hydroxy succinimide (1.20 g, 10.5 mmol) and triethylamine (1.01 g = 1.40 ml, 10 mmol) are dissolved in a mixture dimethylformamide (15 ml) and acetonitrile (15 ml). The solution is cooled to -10°C and dicyclohexylcarbodiimide (2.06 g, 10 mmol) is added. The reaction mixture is stored at -10° overnight. The precipitate, N,N'-dicyclohexylurea, is removed by filtration and the solvents evaporated *in vacuo*. The residue is dissolved in a mixture of ethyl acetate (100 ml) and water (50 ml) and the organic phase

G. Peptide Bond Formation with the Aid of Coupling Reagents

extracted with a 10% solution of citric acid in water (50 ml), 0.5 N KHCO_3 (50 ml) and water (50 ml), dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. Toluene (50 ml) is added and removed *in vacuo*. The addition and removal of toluene is repeated and the residue is crystallized from diisopropyl ether [4]. The purified fully blocked tripeptide (6.4 g, 95%) melts at 115–116 °C; $[\alpha]_D^{20} -20.9^\circ$ (*c* 2.8, ethanol). On thin layer chromatograms (silica gel) in the system of *n*-heptane-*tert*.butanol-piperidine (3:1:1) only a single spot can be detected.

1. Wunsch, E., Drees, F.: Chem. Ber. 99, 110 (1966)
2. Wunsch, E., Zwick, A., Wendlberger, G.: Chem. Ber. 100, 173 (1967)
3. It seems to be advisable to filter off the separated *N,N'*-dicyclohexyl-urea prior to the addition of water.
4. Diisopropyl ether is a dangerous material which caused severe explosions. The authors do not recommend its use.

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